FACTORS DETERMINING THE DOSAGE OF PENICILLIN IN THE TREATMENT OF INFECTIONS*

CHARLES H. RAMMELKAMP†

Major, M.C., A.U.S.

WILLIAM M. M. KIRBY‡

Captain, M.C., A.U.S.

IT IS now well established that penicillin is an extremely effective agent for the treatment of many different types of infections, but a review of the clinical reports makes it at once evident that there is little agreement concerning the amount, or the mode or frequency of administration required to effect a satisfactory therapeutic response. Due to the scarcity of the drug there was at first a tendency to administer small amounts, and as a result erroneous impressions arose concerning the effectiveness of penicillin in diseases such as subacute bacterial endocarditis. More recently, doses found arbitrarily to give satisfactory results have been recommended by various groups of investigators. An example of the discrepancies resulting from this procedure is the recommendation, in cases of staphylococcal sepsis treated with a continuous intravenous infusion of penicillin, of 30,000 to 40,000 units daily by one group,[1] while in the opinion of others 300,000 to 400,000 units should be administered each day.[2]

Now that penicillin is readily available, it is desirable to review the laboratory and clinical experiences which have helped to clarify many of the confusing problems of dosage and mode of administration. The purpose of the present report, therefore, is to bring together the important contributions which provide a basis for rational therapy.

---

* From the laboratories of the Commission on Acute Respiratory Diseases and the Medical Service, Regional Hospital #2, Fort Bragg, N. C.

Presented before the Stated Meeting of The New York Academy of Medicine and the Section of Medicine, May 3, 1945.

† Member of the Commission on Acute Respiratory Diseases, Board for the Investigation of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army, Washington, D. C.

‡ Member of staff of Medical Service, Regional Hospital #2, Fort Bragg, N. C.

Two outstanding characteristics of penicillin are its high degree of antibacterial action and its selectivity. In contrast to the sulfonamides whose action is primarily bacteriostatic, penicillin in concentrations obtained in patients causes sterilization of cultures of susceptible organisms within 24 hours and is for this reason often referred to as a bactericidal agent. Unlike true bactericidal substances such as the phenols, which sterilize cultures within a few minutes, penicillin even in high concentrations does not cause death of microörganisms unless the contact is maintained for many hours.[3] Of great practical importance, however, is the fact that both *in vitro* and *in vivo* penicillin is much more effective in its antibacterial action than the sulfonamide drugs.

Selectivity refers to the high degree of antibacterial activity obtained by penicillin against gram-positive bacteria, and its failure to inhibit the growth of the majority of gram-negative bacteria.

Variations in susceptibility to penicillin occur not only among different species of bacteria, but also among various strains of any one species. With most penicillin sensitive bacteria, however, and particularly with the pneumococcus,[4] beta-hemolytic streptococcus,[5] and gonococcus,[6] these variations are not of clinical significance since they are not of sufficient magnitude to affect the results of therapy. For this reason, routine sensitivity tests of the infecting organisms are of no practical value to the clinician in most diseases for which penicillin is employed. There are exceptions, however; for example in certain staphylococcal infections, and in patients with subacute bacterial endocarditis in which the etiological organisms may be penicillin resistant streptococci (enterococci), knowledge of the sensitivity of the organism may be of importance both from the standpoint of therapy and prognosis.

Correlation between the *in vitro* sensitivity of various organisms and clinical results obtained in patients is much closer with penicillin than with the sulfonamides. This is probably due to the fact that substances such as pus and exudate which inhibit the sulfonamides, do not impair the action of penicillin.[7] Indeed, under certain conditions, by promoting the multiplication of bacteria these substances actually seem to enhance the antibacterial action of penicillin.[8]

In view of the frequency with which various organisms acquire resistance to the sulfonamides, the problem of penicillin resistance has received intensive study. Laboratory investigations indicate that re-

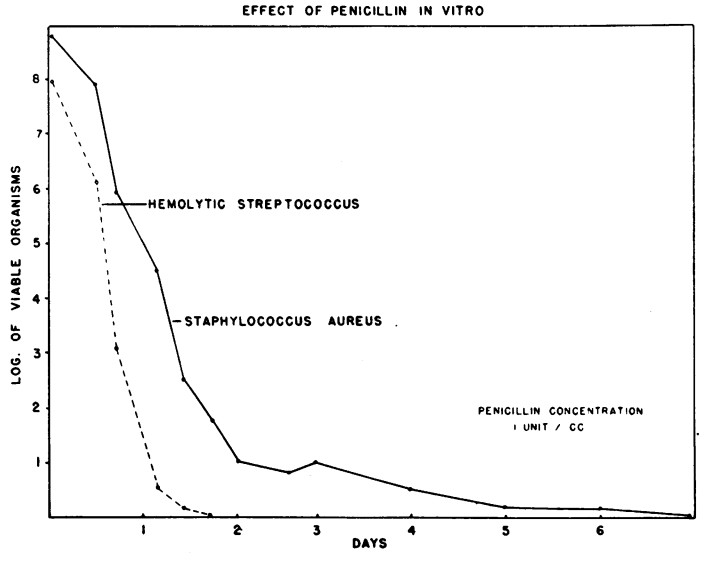


FIG. 1

sistance of the beta-hemolytic streptococcus,[9, 10] gonococcus,[11] pneumococcus,[10] and most other susceptible organisms develops only after prolonged exposure to penicillin. Clinical experience has confirmed these observations; with the exception of the staphylococcus, well substantiated instances of penicillin resistance acquired during therapy have not been described. Further, in patients with streptococcal, pneumococcal, and even staphylococcal infections, in whom relapses occurred because of the location of the organisms in relatively avascular areas, penicillin therapy has been continued for many weeks without the development of any measurable degree of penicillin resistance.[12, 13] Final conclusions cannot be drawn, but the evidence so far available suggests that, with the exception of infections caused by the staphylococcus, penicillin resistance will not become an important clinical problem.

The behavior of the staphylococcus when exposed to penicillin differs from that of other organisms. In contrast to the beta-hemolytic streptococcus, for example, whose growth is completely inhibited by penicillin in 24 to 36 hours, viable staphylococci can often be recovered from cultures for as long as 6 or 7 days (see Figure 1). Clinically, the same situation is observed in patients with empyema. In streptococcal and pneumococcal empyemas the administration of intrapleural penicillin for 1 or 2 days causes complete sterilization of the fluid, while in

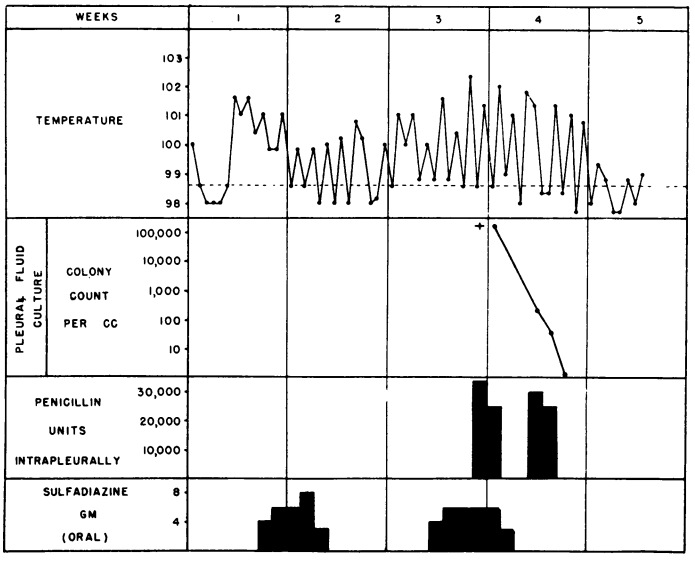


FIG. 2—Antibacterial effect of penicillin in staphylococcal empyema.

staphylococcal infections of the pleural cavity treatment must often be continued for at least a week. An illustrative case of staphylococcal empyema is presented in Figure 2. Twenty-four hours after the initial intrapleural injection of 40,000 units of penicillin the fluid contained 100,000 organisms per cc.; on the 4th day there were 100 organisms per cc.; and sterilization was not effected until the 7th day.

Staphylococci which remain viable for several days in the presence of bacteriostatic concentrations of penicillin are often penicillin-resistant, and contain an enzyme-like substance, "penicillinase", which in small quantities destroys large amounts of penicillin.[14] The relationship of this substance to other "penicillinases" has not been elucidated, but it is probable that studies of various penicillin inactivators will reveal valuable information concerning the basic structure and mode of action of penicillin. Penicillin resistance has been observed in approximately 10 per cent of strains of staphylococci isolated from clinical sources.[9, 15] Whether resistance occurs as an acquired characteristic as a result of contact of the bacteria with penicillin, or whether the sensitive organisms are eliminated leaving naturally resistant staphylococci is not definitely known, but recent studies of two independent investigators[16, 17] support the latter possibility. The evidence further suggests that re-

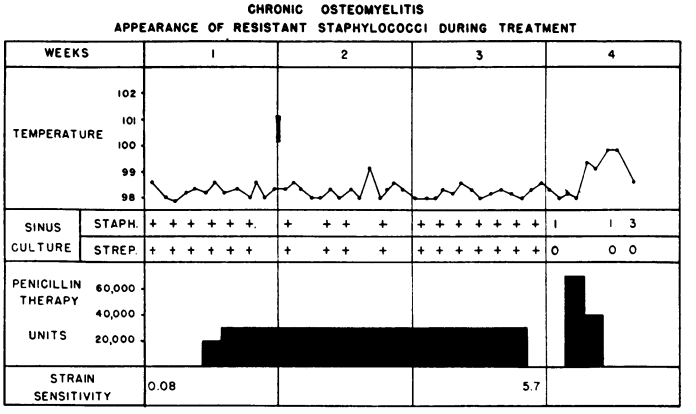


FIG. 3

sistance is much less likely to be manifested if the organisms are, from the start, kept in contact with high concentrations of penicillin. The appearance of penicillin resistance in a case of staphylococcal osteomyelitis treated with inadequate doses of penicillin is presented in Figure 3. The staphylococci isolated prior to the institution of therapy were killed by relatively low concentrations of penicillin, whereas strains isolated after two weeks of treatment with penicillin in a dose of 5,000 units every 4 hours were markedly resistant. More favorable results have been obtained in other cases receiving large doses of penicillin. Here, of course, as in staphylococcal infections in general, the necessity of prolonged therapy as well as adequate doses should be emphasized.

ABSORPTION AND EXCRETION OF PENICILLIN AND ITS DIFFUSION INTO VARIOUS BODY FLUIDS

Before considering the relationship of the concentration of penicillin in the blood to antibacterial action, it is well to review briefly studies on the absorption, excretion and diffusion of penicillin. Penicillin is most frequently administered intramuscularly, although other routes may be used. When injected into the muscle, absorption is rapid, producing maximal concentrations in the blood within 15 to 20 minutes. Beginning penicillin therapy with an initial intravenous injection is therefore completely unnecessary, although it is a common procedure, presumably as a holdover from sulfonamide medication, in which an initial intravenous injection is often advisable.

Once absorbed, penicillin is eliminated from the body very rapidly.

It was originally thought that approximately 40 per cent of the dose injected was destroyed in the body,[7, 18] but recent evidence indicates that destruction by the tissues is actually slight, and that almost all of a single dose administered intramuscularly or intravenously is excreted in the urine.[19] Eighty per cent appears in the urine within two hours, and after the fourth hour the amount remaining in the body is less than 5 per cent of the original dose. By various methods of studying renal clearance it has been definitely demonstrated that the reason for the rapid elimination of penicillin from the body is that it is excreted both by the glomeruli and the renal tubules.[20, 21]

These two factors, rapid absorption and rapid excretion, are responsible for one of the greatest disadvantages of present day penicillin therapy, namely, the necessity for frequent, parenteral injections. Intensive efforts are being made to perfect methods of delaying the absorption of penicillin, by mixing it with beeswax and peanut oil,[22] by the use of vasoconstrictors,[23] and by the application of cold compresses near the site of injection.[24] Attempts are also being made to delay excretion by combining penicillin with substances of high molecular weight,[25] and by the concomitant administration of other agents, such as para-aminohippuric acid, which compete with penicillin for excretion by the renal tubules.[26] The most promising of these methods at present is the intramuscular injection of penicillin-beeswax mixtures; blood levels are maintained for as long as 24 to 28 hours following the administration of 300,000 units of penicillin in 1 cc. of 5 per cent beeswax and peanut oil.

Methods of administering penicillin by mouth are also being studied. The best evidence at present indicates that none of the enteric coatings, oils, or antacids, so far employed give results better than those obtained with the ingestion of penicillin dissolved in tap water. With this method, five or six times as much penicillin must be administered to produce levels equivalent to those following parenteral injections.[27] The increased commercial production of penicillin, plus the fact that oral preparations do not need to be as highly purified as those used parenterally, may eventually make oral administration feasible even if methods of avoiding the destructive action of the gastric juices or increasing absorption from the intestine are not evolved.

Knowledge concerning the distribution of penicillin in the body is essential for good therapeutics. In general, diffusion of penicillin into various body fluids and cavities occurs irregularly, and to a small extent.

For this reason local therapy as well as parenteral injections have been used in the treatment of infections of pleural, pericardial and joint cavities. Frequently when the infection is well localized, as in empyemas, systemic administration of penicillin is not required, favorable results being obtained by local injections alone.[28]

With the usual therapeutic doses penicillin does not appear in measurable quantities in the spinal fluid of normal individuals, and as a result intrathecal administration is commonly used in patients with infections of the meninges.[29] More recently it has been shown that with very large doses of penicillin small amounts can sometimes be detected in the spinal fluid of normal subjects[30] and in patients with meningitis.[31] Others, however, have found that in patients with meningitis, penicillin appears in the spinal fluid very irregularly and in low concentrations.[32] Although a few patients with meningitis have been cured by parenteral administration alone,[33] clinical experience has shown that others have failed to respond to such therapy[32] and that in some cases bacterial meningitis has actually appeared during the course of parenteral treatment of extrameningeal infections.[34]

The subject is controversial, but the best evidence indicates that, for adequate and conservative therapy of staphylococcal, streptococcal and pneumococcal meningitis, both parenteral and intrathecal routes should be used. Since some strains of meningococci are relatively resistant to penicillin[35] and the results obtained with the sulfonamides are so favorable, the latter drugs should be used in the treatment of meningococcal meningitis. The one exception to the use of both intrathecal and parenteral injections of penicillin is syphilitic meningitis, in which results with intramuscular injections alone appear to be excellent.[36]

There are certan conditions, notably subacute bacterial endocarditis, various forms of late syphilis, and chronic osteomyelitis, in which another important factor must be considered, namely, penetration. Early results of penicillin therapy in subacute bacterial endocarditis, using only 5000 units every four hours, were so disappointing that for a time penicillin was abandoned for the treatment of this disease. More recently, large amounts have been administered for two to eight weeks, and a fairly large number of cases have now been reported in which the infection has been eradicated. These favorable results are presumably due to the fact that with the maintenance of high, continuous blood levels of penicillin, there is penetration into the vegetations and destruction of

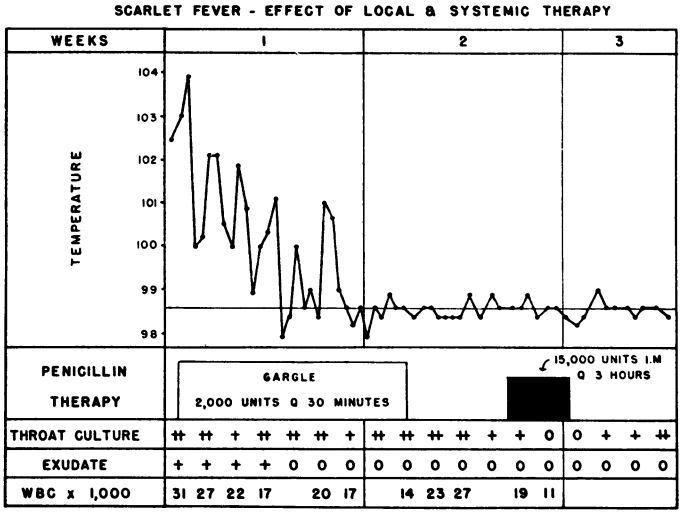


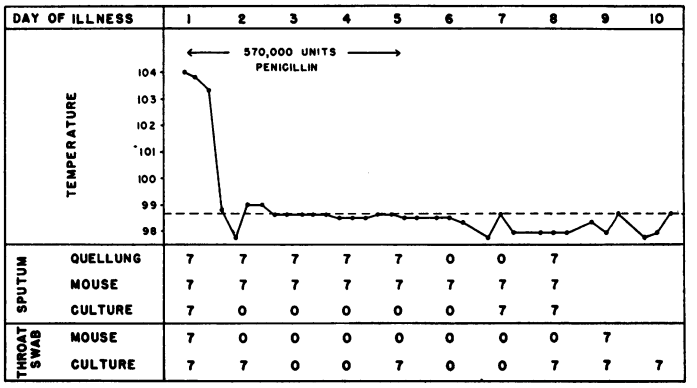
FIG. 4

organisms so that a clinical relapse is prevented. Similarly, although surgical removal of avascular areas is usually necessary in cases of chronic osteomyelitis, instances do occur in which high continuous levels of penicillin produce sufficient penetration to bring about clinical cures.[13]

The failure of penicillin to appear in the saliva of normal subjects[18] or in the sputum of patients with pneumonia[37] may be of importance in infections of the respiratory tract. A study undertaken by the Commission on Acute Respiratory Diseases to determine the effect of penicillin on the normal throat flora showed that the parenteral injection of 20,000 units every 3 hours resulted in no distinct change in the ease of isolation of beta-hemolytic streptococci, pneumococci or staphylococci from the throat. Whether this failure to eradicate organisms from the throats of normal carriers was due to (1) a failure of penicillin to cross the normal mucous membranes, (2) lack of secretion of the drug in the saliva or (3) that the susceptible organisms were present in a resting state and were therefore not susceptible to penicillin, cannot be determined.

In contrast, penicillin is highly effective against organisms in the throat of patients with tonsillitis and pharyngitis. The beneficial effects, however, are exerted only when penicillin is administered parenterally. This is well illustrated in Figure 4, in a patient treated initially with penicillin gargles in whom there was no improvement either clinically or

EFFECT OF PENICILLIN THERAPY IN PNEUMOCOCCAL PNEUMONIA



DAY OF ILLNESS		1	2	3	4	5	6	7	8	9	10
SPUTUM	QUELLUNG	7	7	7	7	7	0	0	7		
	MOUSE	7	7	7	7	7	7	7	7		
	CULTURE	7	0	0	0	0	0	7	7		
THROAT SWAB	MOUSE	7	0	0	0	0	0	0	0	7	
	CULTURE	7	7	0	0	7	0	0	7	7	7

FIG. 5

bacteriologically. The failure to obtain a favorable response by local applications in this instance was undoubtedly due to the fact that the contact between the organism and penicillin was very brief, and emphasizes the point that relatively prolonged contact is necessary. When penicillin was administered by intramuscular injection, however, the streptococci rapidly disappeared, only to reappear when treatment was discontinued after three days. It has been definitely demonstrated that in order to bring about a permanent cure in cases of streptococcal sore throat, it is necessary to continue treatment for a period of at least five days.[38]

In pneumococcal pneumonia, studies by the Commission on Acute Respiratory Diseases have shown that viable, virulent pneumococci can be recovered from the sputum during and following therapy with penicillin for as long as sputum can be obtained.[37] A representative case, showing the method of study, is presented in Figure 5. Thus, although penicillin may destroy some of the organisms present in the parenchyma of the lung, a reactivation of the infection may be caused by pneumococci remaining viable in the bronchial exudate if treatment is discontinued too soon. Clinical relapses occurring in patients treated with penicillin for only two or three days have been described by Tillett, Cambier, and McCormack.[28]

With both streptococcal pharyngitis and pneumococcal pneumonia, then, it is necessary to continue treatment for several days to insure sterilization of the infected tissues and prevent relapses. It would appear that penicillin merely inhibits growth of the bacteria, thereby localizing

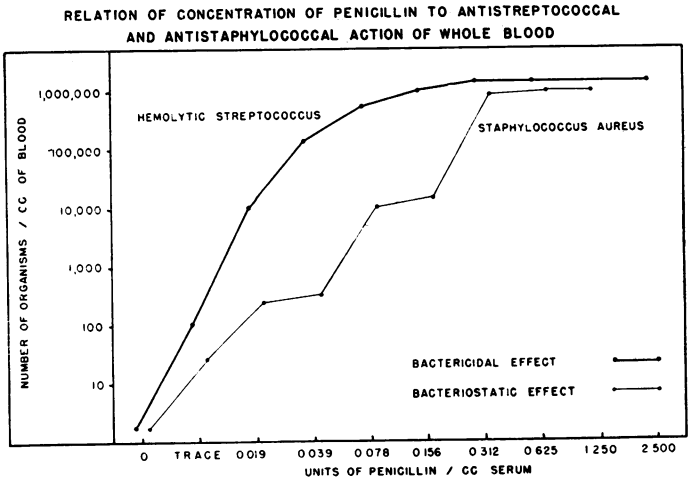


FIG. 6

the infection; actual recovery is brought about by the body's own defense mechanisms. The development of natural immunity is probably of importance also in conditions other than pneumonia and pharyngitis, but so far these relationships have not been worked out in detail.

RELATION OF BLOOD CONCENTRATION TO ANTIBACTERIAL ACTIVITY

Ideally, the objective of therapy with penicillin, as with other chemotherapeutic agents, should be to maintain, at the site of the infection, concentrations of penicillin which exert maximal antibacterial activity throughout the entire period of treatment. From a practical standpoint this is not feasible at the present time, and is even considered disadvantageous by some observers,[39] on the basis that since penicillin is effective against bacteria only during the stage of multiplication, intermittent therapy will allow periods of multiplication alternating with periods of bacteriostasis, and will therefore be more effective. This objection to continuous therapy, based on *in vitro* observations, does not take into consideration conditions existing locally in the tissues, and from a practical standpoint the results obtained clinically with continuous effective concentrations of penicillin in the blood stream are excellent. Indications are that in the future penicillin will be administered by methods which will provide constant measurable blood levels.

In vitro tests of whole blood obtained from normal individuals receiving penicillin have shown[40] maximal antibacterial activity against

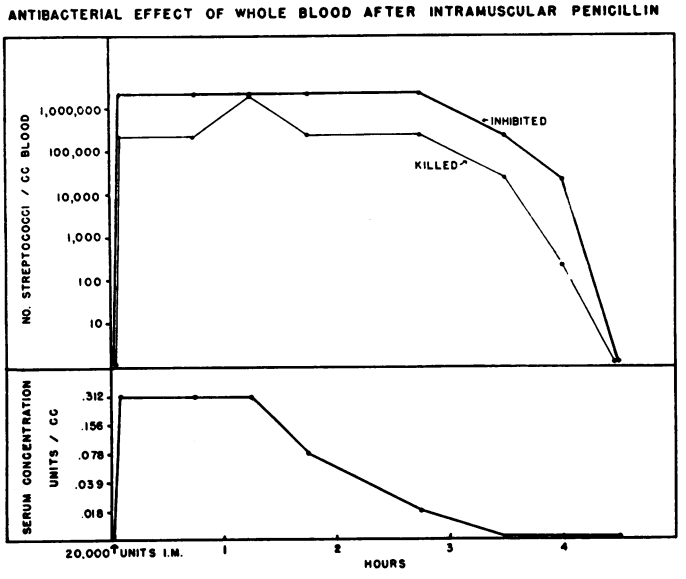


FIG. 7

hemolytic streptococci and staphylococci by concentrations as low as 0.04 and 0.2 units per cubic centimeter respectively (Figure 6). Lower concentrations exhibit definite, but not maximal activity; higher concentrations do not produce any appreciable increase in antibacterial action. These values, 0.04 and 0.2 units per cubic centimeter, which apply respectively to a highly sensitive and relatively resistant organism, are of fundamental importance in relation to the concentrations obtained in the blood stream of patients.

Since chemical methods are not available, penicillin assay methods in use at the present time are based on the ability of penicillin to inhibit the growth of sensitive microörganisms. One of the defects of these methods is their inability to detect small concentrations of penicillin in body fluids. The smallest concentrations which can be measured accurately, 0.02 to 0.04 units per cubic centimeter, are highly effective against most penicillin sensitive bacteria, and it is important, therefore, to have some knowledge of how long penicillin exerts therapeutic action in the body after it can no longer be detected in the blood stream.

Bactericidal tests of whole blood obtained from patients during and following therapy have revealed valuable information on this subject.[40] In patients receiving intramuscular injections of 20,000 units, the bac-

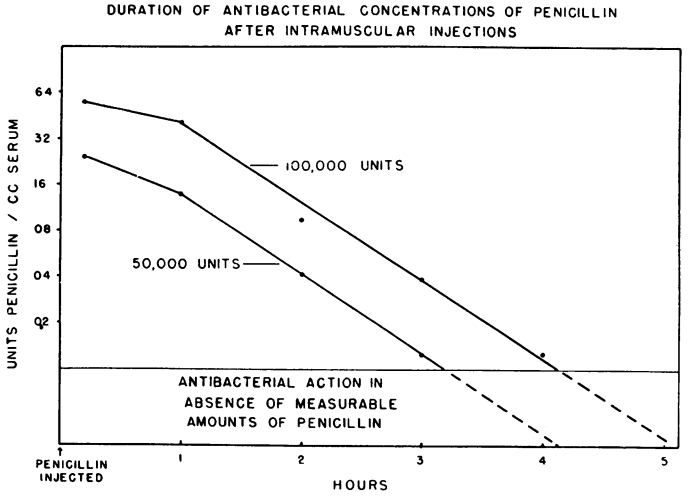


FIG. 8

tericidal power of the blood was found to be increased for a period of from 30 to 75 minutes after penicillin was no longer detectable in the serum by routine assay methods. This is illustrated in Figure 7. From this evidence, it would seem highly unlikely that, following the usual injection of 15,000 to 20,000 units, one could count on any therapeutic activity for more than one hour after detectable levels were no longer present. Since with these doses penicillin is present in measurable amounts in the blood stream for from two to three hours, it can reasonably be assumed that, for all practical purposes, therapeutic activity will cease between the third and fourth hour. These observations are supported by the evidence alluded to previously, indicating that by the end of the fourth hour all but about 5 per cent of the dose administered is excreted in the urine.

A point of interest in this connection is the effect of an increase in the size of the dose upon the duration of therapeutic activity in the blood stream. This is illustrated in Figure 8, in five patients who received single intramuscular injections of 50,000 units, and at a later date additional injections of 100,000 units. Doubling the size of the dose produced blood concentrations twice as high as those observed with 50,000 units, but the duration of assayable blood levels was prolonged by only about one third. This relative inefficiency of larger doses, also pointed out by Fleming,[41] has the additional disadvantage of producing

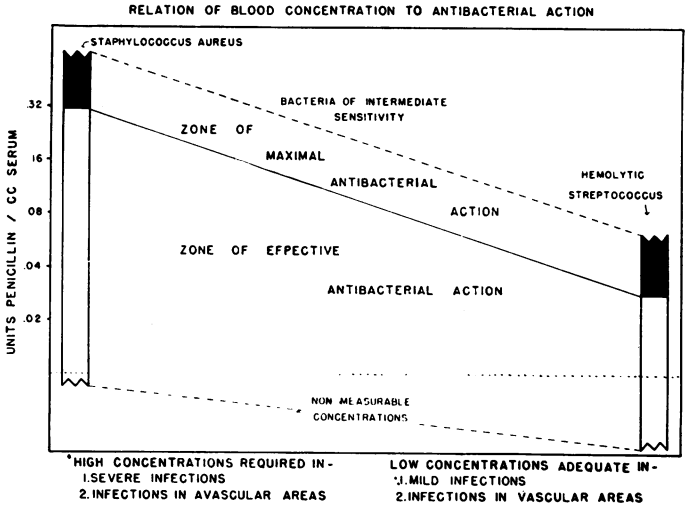


FIG. 9

levels in the blood stream which are considerably in excess of those necessary to exert maximal bacteriostasis.

The broad relationships of the concentration of penicillin in the blood stream to the sensitivity of various susceptible organisms, as well as to other factors, are summarized diagrammatically in Figure 9. The extremes of variation in susceptibility are represented by the staphylococcus and the hemolytic streptococcus. In addition to the staphylococcus, many strains of Streptococcus viridans and non-hemolytic streptococci should be included in the resistant group. Because of the serious nature of infections such as syphilis, diphtheria, and gas gangrene, the organism causing these diseases should also be regarded as highly resistant from a therapeutic standpoint until more information is available concerning their susceptibility both *in vitro* and *in vivo*. Organisms other than the hemolytic streptococcus which may be considered highly sensitive are the gonococcus and pneumococcus. Strains of intermediate sensitivity are represented by the meningococcus. The relative susceptibility of various other organisms, including fungi, spirochetes, and rickettsiae are not definitely known.

Other factors of importance in determining the size of the dose of penicillin are the severity of the infection, the vascularity of the area involved, and the barriers through which penicillin must diffuse or penetrate in order to come into contact with the infecting organisms.

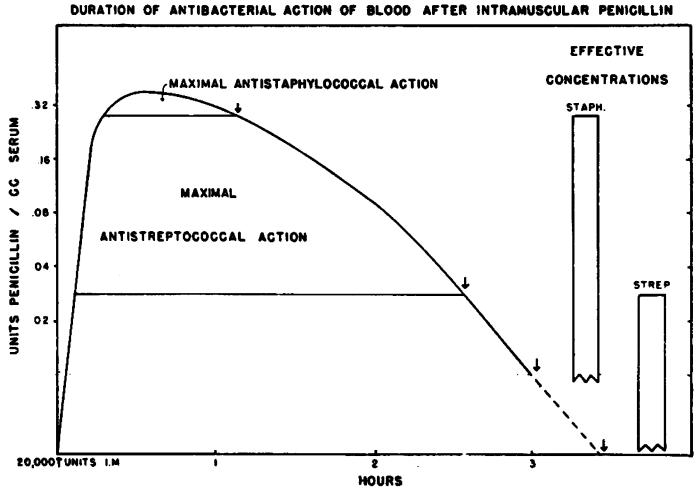


FIG. 10—Arrows indicate time that subsequent injections of penicillin are required to maintain the indicated degree of antibacterial action.

The zone of maximal antibacterial action includes concentrations of penicillin which will cause complete inhibition of growth of the bacteria, and which should ideally be maintained throughout the entire period of treatment. However, definite bacteriostasis is exerted by concentrations considerably lower than those regarded as maximal. As indicated on the chart (Figure 9), this zone of effective antibacterial action includes, for highly susceptible organisms, concentrations of penicillin lower than those which are measurable by present methods of assay.

The relationship of these zones of antibacterial activity to blood levels obtained with intramuscular injections of penicillin in saline is presented in Figure 10. An injection of 20,000 units produces levels in the blood stream considered maximal against the staphylococcus for a period of less than one hour, and levels which are definitely bacteriostatic, although not maximal, for another two hours. For the hemolytic streptococcus, on the other hand, the same dose of penicillin produces concentrations having maximal antistreptococcal action for a period of more than two and one-half hours, with partially inhibitory levels for another one and one-half to two hours.

From these figures it is possible to plan rational schemes of therapy for various infections taking into consideration in addition the factors presented in Figure 9. For staphylococcal infections, for example, injections of 25,000 units every two hours will produce optimal continu-

ous levels in the blood stream; for streptococcal infections, 15,000 units every three hours are adequate. It is not within the scope of this paper to make specific recommendations of dosages for various types of infections; the examples cited are presented merely to illustrate the manner in which the dosage schedules are evolved.

The blood concentrations listed in Figures 9 and 10 may also be attained by continuous subcutaneous, intramuscular, or intravenous infusions of penicillin. Although somewhat tedious, these methods of administration are often desirable for the initial treatment of patients seriously ill from overwhelming infections. Blood levels which can be more or less constantly maintained with a continuous intravenous infusion for twenty-four hours are as follows: with 100,000 units, 0.1 unit per cubic centimeter, with 200,000 units, 0.2 unit per cubic centimeter, and with 400,000 units, 0.4 unit per cubic centimeter.[21] With a continuous intramuscular infusion, the levels are almost identical with those obtained by the intravenous route.[34] Subcutaneous administration, however, produces concentrations only about one half as great as those observed with the other two methods.[21] Daily dosages adequate for various types of infections may be ascertained by reference to Figure 9.

Two points require special emphasis. One is that doses producing optimal concentrations of penicillin in the blood stream are all that are necessary. Giving larger doses merely raises the concentration of penicillin, without increasing the already maximal antibacterial activity. This point is reiterated with the hope that it will temper the present tendency towards overdosage which, while not harmful to the patient, is wasteful and unnecessary.

The other point is that, for all practical purposes, penicillin remains active in the tissues for only three or four hours following the usual therapeutic doses. Penicillin is often administered only four times a day, leaving a period of twelve hours during which no injections are given. Results obtained with this regimen are so favorable in diseases such as pneumococcal pneumonia[28] that there is considerable speculation about the possibility of antibacterial activity remaining in the tissues for many hours after penicillin has apparently left the blood stream. From the data presented in this paper it would appear that this assumption, the implications of which are potentially dangerous, is entirely erroneous.

In conclusion, it must be stated that the exact blood concentrations presented in Figures 9 and 10 cannot be regarded as final from a thera-

peutic standpoint since they are based largely on laboratory observations. Certain factors existing in the tissues, such as the activity of the leukocytes, and the various growth phases of bacteria, are poorly understood, and may eventually alter some of our present concepts. Extensive studies, both clinical and laboratory, will be necessary before a final answer can be reached. For the present, however, the data which are presented are based on sound fundamental observations, and provide a basis for the clinical administration of penicillin which is essentially conservative and rational.

REFERENCES

1. Herrell, W. E. The clinical use of penicillin, an antibacterial agent of biologic origin, *J.A.M.A.*, 1944, *124*:622.
2. Bloomfield, A. L., Rantz, L. A. and Kirby, W. M. M. The clinical use of penicillin, *J.A.M.A.*, 1944, *124*:627.
3. Dubos, R. J. Antimicrobial agents of biologic origin, *J.A.M.A.*, 1944, *124*:633.
4. Robinson, H. J. Toxicity and efficacy of penicillin, *J. Pharmacol. & Exper. Therap.*, 1943, *77*:70.
5. Watson, R. F. Sensitivity of various serological (Lancefield) groups of streptococci to penicillin, *Proc. Soc. Exper. Biol. & Med.*, 1944, *57*:65.
6. Cohn, A. and Seijo, I. H. The *in vitro* effect of penicillin on sulfonamide resistant and sulfonamide susceptible strains of gonococci, *J.A.M.A.*, 1944, *124*:1125.
7. Abraham, E. P. *et al.* Further observations on penicillin, *Lancet*, 1941, *2*:177.
8. Hobby, G. L., Meyer, K., Chaffee, E. and Dawson, M. H. The nature and action of penicillin, *J. Bact.*, 1943, *45*:65.
9. Rammelkamp, C. H. and Maxon, T. Resistance of *Staphylococcus aureus* to the action of penicillin, *Proc. Soc. Exper. Biol. & Med.*, 1942, *51*:386.
10. McKee, C. M. and Houck, C. L. Induced resistance to penicillin of cultures of staphylococci, pneumococci and streptococci, *Proc. Soc. Exper. Biol. & Med.*, 1943, *53*:33.
11. Bahn, J. M., Ackerman, H. and Carpenter, C. M. Development *in vitro* of penicillin-resistant strains of the gonococcus, *Proc. Soc. Exper. Biol. & Med.*, 1945, *58*:21.
12. Bloomfield, A. L., Kirby, W. M. M. and Armstrong, C. D. Study of "penicillin failures," *J.A.M.A.*, 1944, *126*:685.
13. Anderson, D. G., Howard, L. G. and Rammelkamp, C. H. Penicillin in treatment of chronic osteomyelitis, *Arch. Surg.*, 1944, *49*:245.
14. Kirby, W. M. M. Extraction of highly potent penicillin inactivator from penicillin resistant staphylococci, *Science*, 1944, *99*:452.
15. Spink, W. W., Ferris, V. and Vivino, J. J. Comparative *in vitro* resistance of staphylococci to penicillin and to sodium sulfathiazole, *Proc. Soc. Exper. Biol. & Med.*, 1944, *55*:207.
16. Demerec, M. Production of *Staphylococcus aureus* strains resistant to various concentrations of penicillin, *Proc. Nat. Acad. Sc.*, 1945, *31*:16.
17. Kirby, W. M. M. Bacteriostatic and lytic action of penicillin on sensitive and resistant staphylococci, *J. Clin. Investigation*, 1945, *24*:165.
18. Rammelkamp, C. H. and Keefer, C. S. The absorption, excretion, and distribution of penicillin, *J. Clin. Investigation*, 1943, *22*:425.
19. Martin, S. P. and Kirby, W. M. M. Excretion of penicillin following single parenteral injections, *to be published.*
20. Rammelkamp, C. H. and Bradley, S. W. Excretion of penicillin in man, *Proc. Soc. Exper. Biol. & Med.*, 1943, *53*:30.
21. Rantz, L. A. and Kirby, W. M. M. The absorption and excretion of penicillin following continuous intravenous and

subcutaneous administration, *J. Clin. Investigation,* 1944, *23*:789.

22. Romansky, M. J. and Rittman, G. E. Method of prolonging action of penicillin, *Science,* 1944, *100*:196.
23. Fisk, R. T., Foord, A. G. and Alles, G. Prolongation of penicillin activity by means of adrenalin, *Science,* 1945, *101*:124.
24. Trumper, M. and Hutter, A. M. Prolonging effective penicillin action, *Science,* 1944, *100*:432.
25. Chow, B. F. and McKee, C. M. Interaction between crystalline penicillin and human plasma proteins, *Science,* 1945, *101*:67.
26. Beyer, K. H., Flippin, H., Verwey, W. F. and Woodward, R. Effect of para-aminohippuric acid on plasma concentration of penicillin in man, *J.A.M.A.,* 1944, *126*:1007.
27. McDermott, W., Bunn, P. A., Benoit, M., DuBois, R. and Haynes, W. Oral penicillin, *Science,* 1945, *101*:228.
28. Tillett, W. S., Cambier, M. J. and McCormack, J. E. The treatment of lobar pneumonia and pneumococcal empyema with penicillin, *Bull. New York Acad. Med.,* 1944, *20*:142.
29. Rammelkamp, C. H. and Keefer, C. S. The absorption, excretion, and toxicity of penicillin administered by intrathecal injection, *Am. J. M. Sc.,* 1943, *205*:342.
30. McDermott, W., Eagle, H. and Nelson, R. A. *Unpublished observations.*
31. Rosenberg, D. H. and Sylvester, J. C. Excretion of penicillin in spinal fluid in meningitis, *Science,* 1944, *100*:132.
32. Kinsman, J. M. *et al. Unpublished observations.*
33. Price, A. H. and Hodges, J. H. Treatment of meningitis with penicillin injected intravenously and intramuscularly, *New York State J. Med.,* 1944, *44*:2012.
34. Anderson, D. G. The treatment of infections with penicillin, *New England J. Med.,* 1945, *232*:400; 423.
35. Meads, M., Harris, H. W., Samper, B. A. and Finland, M. Treatment of meningococcal meningitis with penicillin, *New England J. Med.,* 1944, *231*:509.
36. Nelson, R. A. and Duncan, L. Acute syphilitic meningitis treated with penicillin, *Bull. Johns Hopkins Hosp.,* 1944, *75*:327.
37. Commission on Acute Respiratory Diseases. *To be published.*
38. Plummer, N., Duerschner, D. R., Warren, H. D., Rogliano, F. T. and Sloan, R. A. Penicillin therapy in hemolytic streptococcic pharyngitis and tonsillitis, *J.A.M.A.,* 1945, *127*:369.
39. Bigger, J. W. Treatment of staphylococcal infections with penicillin by intermittent sterilization, *Lancet,* 1944, *2*:497.
40. Rammelkamp, C. H. and Keefer, C. S. Penicillin, its antibacterial effect in whole blood and serum for the hemolytic streptococcus and Staphylococcus aureus, *J. Clin. Investigation,* 1943, *22*:649.
41. Fleming, A., Young, M. Y., Suchet, J. and Rowe, A. J. E. Penicillin content of blood serum after various doses of penicillin by various routes, *Lancet,* 1944, *2*:621.